PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 143020.6 SB	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/IL 03/00218	International filing date *(day/month/year)* 13.03.2003	Priority date *(day/month/year)* 13.03.2002
International Patent Classification (IPC) or both national classification and IPC A61K31/6615		
Applicant YEDA RESEARCH AND DEVELOPMENT COMPANY LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02.10.2003	23.08.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Elliott, A Telephone No. +49 89 2399-8218

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL 03/00218

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

2, 4, 6-23	as originally filed
1, 3, 3a	filed with telefax on 26.02.2004
5	filed with telefax on 23.05.2004

Claims, Numbers

1-14	filed with telefax on 23.05.2004

Drawings, Sheets

1/10-10/10	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	-

2. Citations and explanations

   see separate sheet

In amended form the application relates to cyclic phosphates as defined by formula I in claim 1, to pharmaceutical compositions comprising said compounds and a pharmaceutically acceptable carrier and to the use of the compounds for the preparation of medicaments for treating disorders and diseases treatable by promoting cell differentiation, e.g. cancer.

The following documents have been taken into consideration:

D1: WO-A-00 57865
D2: Eur. J. Biochem. 2000, 267, 2547-54
D3: J. Org. Chem. 1996, 61, 7633-6
D4: Bioorg. & Med. Chem. Letters 1996, 6(13), 1523-4
D5: J. Org. Chem. 1986, 51, 4310-1
D6: Acta Cryst. 1986, C42, 1462-3
D7: JACS 1980, 102(5), 1665-70
D8: JACS 1980, 102(5), 1655-60
D9: J. Org. Chem. 1980, 45(7), 1282-6
D10: Canadian Journal of Chemistry 1978, 56(18), 2396-404
D11: J. Org. Chem. 1977, 42(13), 2260-4
D12: Lipids 1973, 8(5), 289-94
D13: Phosphorus & related Group V Elements 1973, 2(5-6), 245-8
D14: J. Chem. Soc. 1957, 1109-14
D15: Biochem. J. 1981, 195(1), 301-6
D16: Pharm. Bull. 1957, 5(2), 121-6

Re Item V.

i. Novelty (Article 33(2) PCT)

The way in which the subject-matter of the application has been amended during the present international proceedings (namely the amendment of claim 1 to exclude certain compounds by means of disclaimer) brings about the acknowledgement of novelty for the presently-claimed subject-matter. (The following lack of novelty objections were raised against the claims as originally filed: D1 - compounds I, IV, VI and VIII in appendix A were seen to be novelty-destroying to original claim 1 and their use novelty-destroying to original claims 6-9 and 11-14; D2 - compounds I, III, IV and VI from D2 were seen to fall under the scope of original claim 1; D3 & D4 were seen to disclose two compounds falling under the scope of original claims 1 & 2 (D3 - compounds 7 & 8; D4 - compounds 2 & 6); D5 - compounds 3 & 4 were seen to fall under the scope of original claim 1; D6 - the compound discussed in D6 was seen to fall under the scope of original claim 1; D7 - a number of compounds appearing in the left-hand column on page 1666 of D7 were seen to fall under the scope of original claims 1 and 3 - the phenyl ester of 5-methoxytrimethylenephosphoric acid, 5-

methoxytrimethylenephosphoric acid, 5-ethoxytrimethylenephosphoric acid, 5-isoproxytrimethylene phosphate and 5-tert-butoxytrimethylene phosphate; D8 - 5-methoxytrimethylenephosphoric acid mentioned in D8 was seen to fall under the scope of original claims 1 and 3; D9 - 5-ethoxytrimethylenephosphoric acid discussed in D9 was seen to fall under the scope of original claims 1 and 3; D10 - the cyclic phosphate diester prepared from 1,3-propanediol in Table 2 and the phenyl phosphate triesters prepared from 1,3-propanediol and 2-methoxy-1,3-propanediol in Table 1 of D10 fall under the scope of original claims 1 & 3: D11 - compounds 5 & 6 from D11 were seen as novelty-destroying to original claim 1; D12 - compounds III and VII from D12 were seen as anticipating the subject-matter of original claim 1; D13 - compound 3 from D13 was seen as novelty-destroying to claim 1 and compound 4 as novelty-destroying to original claims 1 and 2; D14 - compound VII from D14 was seen as novelty-destroying to original claim 1).

ii. Inventive Step (Article 33(3) PCT)

Documents D1 and D2 are to be regarded as closest prior art in the present instance. D1 discloses pharmaceutical compositions comprising cyclic glycerophosphates and analogs thereof for promoting neural cell differentiation. D2 discloses the use of cyclic glycerophosphates and their deoxy analogues in the induction of intracellular signalling; D2 suggests that the compounds disclosed therein may take part in processes associated with cell differentiation. The difference between the subject-matter of the present claims and that of D1 and D2 can be seen in the restrictions to the presently-claimed compounds by proviso.

The object of the present application can be seen to provide further compounds for the treatment of diseases or disorders treatable by cell differentiation therapy.

An inventive step can be acknowledged for the subject-matter of the application in amended form as D1 and D2 do not envisage compounds with the combination of X and X' as not stipulated by proviso for the purpose of treating diseases or disorders treatable by cell differentiation therapy.

Summing up, novelty and inventive step are provisionally acknowledged for claims 1-14, provisionally as a number of inconsistencies have arisen as a result of the newly-introduced provisos (see point VIII below).

Re Item VIII.

Concerning the provisos/disclaimers which are now present in claim 1, the disclaimer "when n=1, X'=H and X=NH-CO-CH_3, Y is not O-p-NO_2-C_6H_4" is disclaiming the compound having the registry number 183-43-7, which appears in the accidental disclosures D3 and D4. The newly-introduced proviso "when X' is hydrogen, X is NHR or NH-CO-R" render the accidental disclosues of documents D5-D16 no longer relevant to the subject-matter of the claims - this proviso is however only to be seen as based on a very small number of exemplified compounds in the present application for the case n=1, e.g. compounds (b) and (c) of claim 5.

With the introduction of the provisos, a number of the preferred compounds under claim 5 no longer fall under the scope of amended claim 1. Also the newly-introduced provisos are in conflict with those in the claim as originally filed.

A further investigation of the provisos/disclaimers is therefore deemed necessary in the regional phase(s) to come.

DERIVATIVES OF 1,3-CYCLIC PROPANDIOL PHOSPHATE AND THEIR ACTION IN DIFFERENTIATION THERAPY

FIELD OF THE INVENTION

This invention relates to 1,3-cyclic propandiol phosphate derivatives, pharmaceutical compositions comprising these derivatives and use thereof as cell stimulants.

PRIOR ART

The following is a list of references which is intended for a better understanding of the background of the present invention.

Boyd, R.K., De Freitas, A.S.W., Hoyle, J., McCulloch, A.W., McInnes, A.G., Rogerson, A. and Walter, J.A., *J. Biol. Chem.*, 262:12406-12408 (1987).

Clarke, N. and Dawson, R.M.C., *Biochem. J.*, 153:745-747 (1976).

Dawson, R.M.C., *Ann. Rept. Progr. Chem.* 55:365, (1958).

Dawson, R.M.C., Freinkel, N., Jungalwala, F.B. and Clarke, N., *Biochem. J.*, 122:605-607, (1971).

Forrest, H.S. and Todd, A.R., *J. Chem. Soc.*, 1950, 3925, (1950).

Friedman, P., Haimovitz, R., Markman, O., Roberts, M.F. and Shinitzky, M., *J. Biol. Chem.*, 271:953-957 (1996).

Kennedy and Weiss, *J. Biol. Chem.*, 222:193 (1956).

Kurokawa, H, Lenferink, AE, Simpson, JF, Pisacane, PI, Sliwkowski, MX, Forbes, JT, Arteaga, CL (2000) Cancer Res 60: 5887

Leloir, L.F., *Biochem. Biophys., J.*, 33:186 (1951).

Markham, R. and Smith, J.D., *Biochem. J.*, 52:552- (1952).

myoinositol-1,2-cyclic phosphodiester (Dawson *et al.*, 1971) and cyclic lysophosphatidic acid (Friedman *et al.*, 1996). Synthesis of di- and tri-esters of 1,3-Cyclic phosphates, having biological interest, was disclosed by Penney, C.L. & Belleau, B. in *Can. J. Chem.* (1978) **56**, 2396-2404. Derivatives of 1,3-cyclic phosphates trimesters were used as transition state analogues in the construction of catalytic antibodies (Lavey, B.J. & Janda, K.D. in *J. Org. Chem.* (1986) **61**, 7633-7636 and in *Bioorganic & Medi. Chem. Letts.* (1996) **6**, 1523-24). The crystallographic structures of 5-ethoxytrimethylenephosphoric acid (Gerlt, J.A. et al. J. Org. Chem. (1980) 45, 1282-1286), 1,3,2-Dioxaphosphrinanes (Jones, A.S et al. *J. Org. Chem.* (1986) **51**, 4310-4311) and 5-hydroxy-2-methoxy-1,3,2λ^5-dioxaphosphacyclohexane-2-oxide (Hamor, T.A. *Acta Cryst.* (1986) **C42**, 1462-1463) were reported. The conformational properties of 5-alkoxy and 5-alkyl substituted trimethylene phosphates in solution (Gerlt, J.A. et al. *J. Am. Chem. Soc.* (1980) **102**, 1665-1670) and the thermochemical identification of 3', 5'-cyclic nucleotides, in particular 2-alkoxy derivatives of 1,3-cyclic glycerophosphates (Gerlt, J.A. et al. *J. Am. Chem. Soc.* (1980) **102**, 1655-1660) were reported. Displacement reaction of 1,3-cyclic glycerophosphates have also been reported (Baran, J.S et al. *J. Org. Chem.* (1977) **42**, 2260-2264). Preparation and chemistry of sn-glycerol-cyclic-phosphodiester isomers (Buchnea, D. *Lipids* (1973) **8**, 289-294) and 2,6,7-trioxa-1-phosphabicyclo[2,2,1]heptane (Denney, D.B. & Varga, S.L. *Phosphorous* (1973) **2**, 245-248) were also published.

Except for cyclic AMP and cyclic GMP, which have been extensively studied, no specific biological activities have been so far assigned to the other biological cyclic phosphates.

Breast cancer cells in their virulent undifferentiated state are characterized by lack of functional estrogen and/or progesterone receptors. To date, no method for *in situ* differentiation of breast cancer cells has yet been proven effective in patients.

GLOSSARY

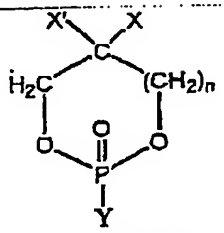
The following is an explanation of some terms used above and in the following description and claims:

CPP – the 1,3-cyclic propandiol phosphates derivatives used in the present invention.

Target cells – any cells, which have the potential to mature into neural cells. Non-limiting examples of such cells are MCF-7 and T47D human breast cancer cells.

Substantially maintaining - this term relates to the capability of analogs to promote the activity carried out by the cyclic glycerophosphate from which they were derived to a certain extent. The analog's activity will be considered to be substantially maintained wherein the activity is 30% or above, preferably 50% or above, more preferably 70% or above, and most preferably 90% or above the level of the activity of the cyclic glycerophosphate.

Effective amount – wherein the method of the invention is intended for prevention of a non-desired condition, the term *"effective amount"* should then be



formula I

or pharmaceutically acceptable salts thereof,

wherein

n is 0 or 1;

X is hydrogen, O-R, NH-R or N-(C=O)-R;

X' is hydrogen or CH_2OH;

Y is O-R_1, NH-R_1;

R is hydrogen, linear or branched alkyl, linear or branched acyl, substituted or non-substituted aryl or araalkyl residue;

R_1 is hydrogen, linear or branched alkyl, linear or branched acyl, substituted or non-substituted aryl, alkylcarboxy ester or alkyl-N-R_2R_3;

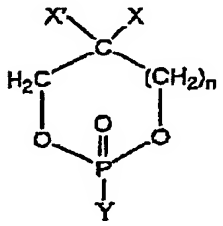
R_2 and R_3 are independently hydrogen or an alkyl group;

provided that when X and X' are hydrogen Y is not OR_1 wherein R_1 is hydrogen, alkyl or aryl; that when X' is hydrogen X is NHR or N(C=O)-R; provided that when X' is CH_2OH then X is NH-R or NO_2; and that when n=1, X'=H and X= NH(C=O)-CH_3, Y is not O-p-NO_2-C_6H_4.

As used herein the term *"alkyl"* refers to an alkyl group having from 1 to 24 carbon atoms, e.g. preferably from 3 carbon atoms to 20 carbon atoms, most preferably from 5 carbon atoms to 15 carbon atoms; the term *"acyl"* refers to an aliphatic saturated or unsaturated C_1 - C_{24} acyl group, preferably an acyl group having an even number of carbon atoms, most preferably an acyl group derived from a natural fatty acid such as a saturated aliphatic acyl group selected from acetyl, butyryl, caproyl, octanoyl, decanoyl, lauroyl, myristyl, palmitoyl and stearoyl, or an unsaturated aliphatic acyl group selected from palmitoleyl, oleyl, linoleyl, and ricinoleyl; and the term *"aryl"* refers to a mono- or poly-carbocyclic

CLAIMS:

1. A compound of the following formula I:



or pharmaceutically acceptable salts thereof,

wherein:

n is 0 or 1;

X is hydrogen, O-R, NH-R or N-(C=O)-R;

X' is hydrogen or CH_2OH;

Y is O-R_1, NH-R_1;

R is hydrogen, linear or branched alkyl, linear or branched acyl, substituted or non-substituted aryl or araalkyl residue;

R_1 is hydrogen, linear or branched alkyl, linear or branched acyl, substituted or non-substiuted aryl, alkylcarboxy ester or alkyl-N-R_2R_3;

R_2 and R_3 are independently hydrogen or an alkyl group;

alkyl is an alkyl group having from 1 to 24 carbon atoms, preferably from 3 carbon atoms to 20 carbon atoms, most preferably from 5 carbon atoms to 15 carbon atoms;

acyl is an aliphatic saturated or unsaturated C_1 - C_{24} acyl group, preferably an acyl group having an even number of carbon atoms, and most preferably an acyl group derived from a natural fatty acid such as a saturated aliphatic acyl group or an unsaturated aliphatic acyl group;

aryl is a to a mono- or poly-carbocyclic aryl group, most preferably phenyl, optionally substituted by C_1 - C_4 alkyl, halogen and/or hydroxy;

provided that when X and X' are hydrogen Y is not OR_1 wherein R_1 is hydrogen, alkyl or aryl; that when X' is hydrogen X is NHR or N(C=O)-R; provided that when X' is CH_2OH then X is NH-R or NO_2; and that when n=1, X'=H and X= $NH(C=O)-CH_3$, Y is not O-p-NO_2-C_6H_4.

2. A compound according to claim 1, wherein the acyl moiety is selected from the group comprising of acetyl, butyryl, caproyl, octanoyl, decanoyl, lauroyl, myristyl, palmitoyl and stearoyl, palmitoleyl, oleyl, linoleyl, and ricinoleyl.

3. A compound according to claim 1 wherein Y is OH and X is O-R or NH-R; wherein R is a linear or branched alkyl or linear or branched acyl.

4. A compound according to claim 1 wherein X is hydrogen and Y is O-acyl or NH-R_1; wherein R_1 is a linear or branched alkyl or linear or branched acyl.

5. Compounds of formula I according to claim 1 selected from the group consisting of:

(a) 1,3-cyclic propandiol phosphate-5-oleoyl;

(b) 1,3-cyclic propandiol phosphate-5- benzyloxy;

(c) 1,3-cyclic propandiol phosphate-5- benzylamino;

(d) 1,3-cyclic propandiol phosphate-5- caproylamido;

(e) 1,3-cyclic propandiol phosphate-2-benzyloxy;

(f) 1,3-cyclic propandiol phosphate-2- acetyloxy;

(g) 1,3-cyclic propandiol phosphate-2-methylamino;

(h) 1,3-cyclic propandiol phosphate-5-glycine ethylester;

(i) 2-methyl 1,3-cyclic propanediol phosphate;

(j) 2-dimethylamine ethyl ester 1,3-cyclic propanediol phosphate;

(k) 1,3-cyclic propanediol phosphoamidate;

(l) 1,3-cyclic propanediol N-ethyl phosphoamidate;

(m) 1,3-cyclic propanediol phosphoamidate glycine ethylester;

(n) 2-benzyloxy 1,3-cyclicpropanediol phosphate;

(o) 2-caproimido 1,3-cyclicpropanediol phosphate;

(p) 5-amino-5-hydroxymethyl-2-oxo-2λ5-[1,3,2]dioxaphosphinan-2-ol;

(q) 5-nitro-5-hydroxymethyl-2-oxo-2λ5-[1,3,2]dioxaphosphinan-2-ol;

or pharmaceutically acceptable salts thereof.

6. A pharmaceutical composition comprising a pharmaceutical acceptable carrier and, as an active ingredient, a compound of the general Formula I in Claim 1 or pharmaceutically acceptable salt thereof.

7. A pharmaceutical composition according to claim 6, for promoting cell differentiation in cancerous cells.

8. A pharmaceutical composition according to claim 6, for promoting protein expression in cancerous cells.

9. A pharmaceutical composition according to claim 8, wherein said protein is estrogen receptor -α or progesterone receptor.

10. A pharmaceutical composition according to any one of claims 6 to 9 wherein the compound of formula I is selected from the group consisting of

(a) 1,3-cyclic propandiol phosphate-5-oleoyl;

(b) 1,3-cyclic propandiol phosphate-5- benzyloxy;

(c) 1,3-cyclic propandiol phosphate-5- benzylamino;

(d) 1,3-cyclic propandiol phosphate-5- caproylamido;

(e) 1,3-cyclic propandiol phosphate-2-benzyloxy;

(f) 1,3-cyclic propandiol phosphate-2- acetyloxy;

(g) 1,3-cyclic propandiol phosphate-2-methylamino;

(h) 1,3-cyclic propandiol phosphate-5-glycine ethylester;

(i) 2-methyl 1,3-cyclic propanediol phosphate;

(j) 2-dimethylamine ethyl ester 1,3-cyclic propanediol phosphate;

(k) 1,3-cyclic propanediol phosphoamidate;

(l) 1,3-cyclic propanediol N-ethyl phosphoamidate;

(m) 1,3-cyclic propanediol phosphoamidate glycine ethylester;

(n) 2-benzyloxy 1,3-cyclicpropanediol phosphate;

(o) 2-caproimido 1,3-cyclicpropanediol phosphate;

(p) 5-amino-5-hydroxymethyl-2-oxo-2λ5-[1,3,2]dioxaphosphinan-2-ol;

(q) 5-nitro-5-hydroxymethyl-2-oxo-2λ5-[1,3,2]dioxaphosphinan-2-ol;

or pharmaceutically acceptable salts thereof.

11. Use of a compound of formula I for the preparation of a medicament for treating disorders and diseases, which can be treated by promoting cell differentiation.

12. Use according to claim 11, wherein said disorder is tumor growth.

13. Use of a compound of formula I for the preparation of a medicament for treating disorders and diseases, which can be treated by promoting protein expression.

14. Use according to claim 13, wherein said protein is estrogen receptor-α or progesterone receptor.